



HAL
open science

A multi-omics approach to decipher skeletal muscle from GRMD dogs transplanted with MuStem cells.

Aurélie Lardenois, Florence Robriquet, Candice Babarit, Sabrina Jagot, Thibaut Larcher, Mireille Ledevin, Laurence Dubreil, Karl Rouger, Laétitia Guével

► To cite this version:

Aurélie Lardenois, Florence Robriquet, Candice Babarit, Sabrina Jagot, Thibaut Larcher, et al.. A multi-omics approach to decipher skeletal muscle from GRMD dogs transplanted with MuStem cells.. 5th International congress of Myology, 2016, Lyon, France. , 2016, Myology 2016. hal-01361971

HAL Id: hal-01361971

<https://hal.science/hal-01361971>

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Adult muscle regeneration- #2455

P01- 1- The crustacean *Parhyale hawaiiensis* as a genetically tractable model for muscle regeneration

Marco Grillo (1), Michalis Averof (1)

1. Institut de Genomique Fonctionnelle de Lyon, Lyon, France

Many animals have the ability to regenerate body parts that are lost through injury. Regeneration restores both the number of cells and the diversity of cell types of the lost tissue, by mobilizing specific populations of progenitor cells. It also restores pattern, giving rise to well-proportioned and functional organs that are virtually indistinguishable from those of unharmed animals. In spite of great medical and biological interest in regeneration, the molecular and cellular mechanisms underlying this process are still not fully understood. Although progenitor cells have been identified in some contexts, limited progress has been made in understanding the actual mechanisms of regeneration. Part of the problem in identifying these mechanisms has been the lack of appropriate tools that would allow us to visualize cell behaviour in a live regenerating organ. In this context, we are setting out to establish the amphipod crustacean *Parhyale hawaiiensis* as a genetically tractable model for limb regeneration. *Parhyale* is particularly attractive for this because it combines extensive regenerative abilities with great experimental tractability (live imaging at single cell level, clonal tools, selective cell ablation). Recent work in the lab has focused on muscle regeneration, describing a specific cell population that shares several features with satellite cells, the muscle precursors in vertebrates. These satellite-like cells (SLC), which express the typical satellite cells marker Pax3/7, were shown to participate in muscle regeneration after limb amputation in *Parhyale*, similarly to what has been previously described in vertebrates. In this context, we are developing tools to genetically label and manipulate *Parhyale* SLCs, in order to understand their biological properties (cell-cell interaction, proliferation patterns, transcriptional profile and functional heterogeneity) and their role in muscle regeneration and homeostasis.

Satellite cells, adult muscle regeneration, model animals, live imaging

Adult muscle regeneration- #2563

P01- 2- A multi-omics approach to decipher skeletal muscle from GRMD dogs transplanted with MuStem cells

Aurélie Lardenois (1), Florence Robriquet (1), Candice Babarit (1), Sabrina Jagot (2), Thibaut Larcher (1), Mireille Ledevin (1), Laurence Dubreil (1), Karl Rouger (1), Laetitia Guevel (3)

1. INRA UMR703 PAnTher, LUNAM Université, Oniris, NANTES, France

2. Université de Nantes, NANTES, France

3. INRA UMR703 PAnTher, LUNAM Université, Oniris, Université de Nantes, NANTES, France

Duchenne Muscular Dystrophy (DMD), the most common form of inherited neuromuscular disorder, is caused by mutations in the dystrophin gene leading to the protein lack. Membrane disorganization and subsequent alterations in signaling pathways and energy metabolism play important roles in muscle fibre necrosis. Systemic delivery of allogeneic MuStem cells, which correspond to a skeletal muscle-resident stem cell subset isolated based on initial delayed adhesion properties, generates a muscle tissue remodeling and gives rise to striking clinical benefits in Golden Retriever Muscular Dystrophy (GRMD) dog (Rouger et al., 2011).

To assess the impact of MuStem cell delivery on the skeletal muscle of transplanted GRMD dogs, we combined a transcriptomic study using Agilent gene expression microarrays (Robriquet et al., 2015) with a quantitative proteomic analysis (ICPL/LC-MS/MS) and an exploration of miRNAs expression levels on muscles 6 months after cell transplantation. Finally to get deeper into this exploration we performed an expression profiling using high-throughput sequencing (RNA-seq) on the same samples. This multi-omics approach exhibits highly complementary results. As a major effect of MuStem cell administration, we identified the enhancement of the muscle fibre regeneration in parallel with a global remodeling of fibre type composition as well as an effect on the connection between the muscle fibre and the extracellular matrix. Furthermore, MuStem cell delivery seems to play a role on lipid homeostasis, energy metabolism and could also play a role in a protection against oxidative stress as well as increase the inflammatory response (Lardenois et al., submitted). In addition, we demonstrate an effect of MuStem cell transplantation on the muscle expression of miR-133 and miR-222 (Robriquet et al., submitted). Overall, the combination of data at the transcript, protein and miRNA levels allow to pave the way to the understanding of the consequences of MuStem cell administration on skeletal muscle fibres. This strategy has a great potential to contribute to the identification of therapeutic tissue markers of MuStem cell transplantation and thus represents an interesting tool to monitor therapeutic effects during DMD-dedicated preclinical studies.

Duchenne muscular dystrophy, Cell therapy, MuStem cells, OMIC strategies, tissue biomarker

Adult muscle regeneration- #2620

P01- 3- PABPN1 regulates RNA processing in ageing muscles

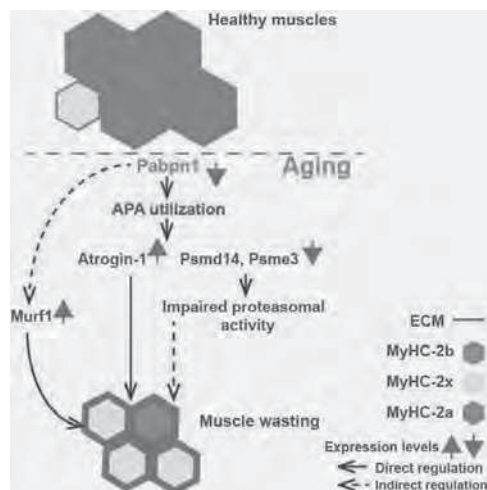
Muhammad Riaz (1), Yotam Raz (1), Vered Raz (2)

1. Leiden University Medical Center, Leiden, Pays-Bas

2. Leiden, Pays-Bas

Expression levels of Poly(A) Binding Protein Nuclear 1 (PABPN1), a multifunctional regulator of mRNA processing, decline specifically in skeletal muscles during aging. An expansion mutation in PABPN1 is the genetic cause of oculopharyngeal muscle dystrophy (OPMD), a late onset and rare myopathy. Reduced Pabpn1 availability correlates with manifestation of muscle symptoms in OPMD. We hypothesized that altered PABPN1 expression is an underlying cause of muscle wasting. To test this, we stably down-regulated Pabpn1 in mouse tibialis anterior muscles by local AAV-mediated transduction of shRNAs. We found that a mild reduction in PABPN1 levels causes alternative polyadenylation site (APA) utilization at the 3'-UTR of transcripts of the ubiquitin-proteasome system and impairs proteasomal activity. Importantly, PABPN1 down-regulation leads to muscle

pathology including myofiber atrophy, extracellular matrix thickening and myofiber-type transitions, which largely resembles aging muscle pathology. We suggest that PABPN1-mediated alternative APA utilization plays a role in aging-associated muscle wasting.



RNA processing, muscle aging, quantitative muscle pathology

Adult muscle regeneration- #2648

P01- 4- Identification of muscle cells expressing aldehyde dehydrogenase in healthy and DMD patients and models, and their changes with ageing

Jessy Etienne (1), Stéphanie Riveron (1), Cyril Catelain (1), Anne-Sophie Pellen (1), Stéphane Vasseur (2), Maud Chapart (2), Stéphanie Lorain (1), Elodie Laffrat (3), Isabel Punzon (4), Inès Barthelemy (4), Stéphane Blot (4), Gillian Butler-Browne (1), Jean-Thomas Vilquin (1)

1. Sorbonne Universités UPMC Univ Paris 06, Inserm UMRS974, CNRS FRE3617, Myology Research Center, Pitié Salpêtrière Hospital, Paris, France

2. MYOBANK AIM, Pitié-Salpêtrière Hospital, Paris, France

3. Sorbonne Universités UPMC Univ Paris 06, Inserm UMR-S975- CNRS UMR 7225, CR-ICM, Pitié-Salpêtrière Hospital, Paris, France

4. Inserm IMRB U955-E10, 94000 Créteil- Ecole nationale vétérinaire d'Alfort (EnvA)- Université Paris-Est Créteil, Maisons-Alfort, France

Aldehyde dehydrogenases (ALDH) constitute a large family of enzymes involved in the detoxification of aldehydes and/or in the metabolism of retinoic acid. They are frequently considered as one stem cell marker. Two subpopulations of ALDH+ cells have been described in human skeletal muscle. In vitro, ALDH+/CD34+ cells show a mesenchymal-like profile with adipogenic and osteogenic differentiation capacities while ALDH+/CD34- cells differentiate towards the myogenic lineage. This is suggesting that the latter population may be involved in muscle homeostasis. To date, there is little information regarding their presence and evolution during ageing or in pathological situations.

We refined the presence and proportions of ALDH+ cells in several skeletal and cardiac muscle groups in non-human primates, whatever their anatomical position or embryologic origin. We also analyzed the presence and evolution of ALDH+ cell populations by flow cytometry using samples from healthy human donors of various ages, or presenting with Duchenne Muscular Dystrophy (DMD). Some extracellular markers were differentially expressed by the populations of ALDH+ cells (CD9, CD10, CD31, CD49s, CD105, CD106, CD140b, CD146, CD184,...). We observed a trend toward a slight decrease of classical endothelial (CD34+/CD31+) and myogenic (CD56+) cells with age, paralleled by the evolution of ALDH+/34+ and ALDH+/34- populations. In DMD patients, despite their young age, the ALDH+/CD34- subpopulation strongly decreased as compared to healthy controls, while the proportion of CD45+ cells (hematopoietic cells, monocytes) increased. Cell cultures prepared from the DMD biopsies yielded far less ALDH+/CD56+ progenitors than healthy controls. The results were confirmed in healthy dogs and in their DMD counterpart (GRMD).

Finally, different ALDH isoenzymes could be associated with commitment and/or differentiation markers in muscle cells. In the tissue, immunohistofluorescence studies highlighted the cells in endomysial position or in contact with vessels depending on isoenzyme expressed.

This study suggests a role played by some ALDH+ cell populations in muscle formation and/or repair that may become promising new tools for therapeutic strategies.

With support of: Association Française contre les Myopathies, Duchenne Parent Project Netherlands, DIM-Stempole Region Ile de France, Ligue contre les Cardiomyopathies, Program ?Investissements d'avenir? ANR-10- IAIHU-06 (non-human primate work).

Aldehyde dehydrogenase, skeletal muscle, Human, Dog, DMD, myogenesis, CD34, CD56

Adult muscle regeneration- #2695

P01- 5- Implication of the pericytes derived Angiopoietin-1 in adult skeletal muscle homeostasis and repair

Zeynab Koumaha (1), Muriel Rigolet (2), Romain Gherardi (3), Peggy Lafuste (4)

1. Biology of the Neuromuscular System, Team 10 group 1, INSERM IMRB U955, Créteil, France

2. Biology of the Neuromuscular System, Team 10 group 2, INSERM IMRB U955, Créteil, France

3. Biology of the Neuromuscular System, Team 10 group 2, INSERM IMRB U955, Créteil, France

4. Biology of the Neuromuscular System, Team 10 group 1, INSERM IMRB U955, Créteil, France

Muscle growth and regeneration following injury, is regulated by the myogenic stem cells, called satellite cells. The satellite cells are located beneath myofiber basement membranes and closely associated with capillary endothelial cells. We previously observed that 90% of capillaries were associated with pericytes in adult mouse and human muscle. We also have shown during post-natal growth that, by promoting post-natal myogenesis through Insulin like-growth factor 1 and stem cell quiescence through Angiopoietin-1, pericytes play a key role in the microvascular niche of satellite cells. Consistently, here we show that in a mouse model of muscle pericytes depletion, the loss of the perivascular cells induces a spontaneous necrosis of the muscle. We also observed after induced chemical injury of the muscle in a mouse model of deletion of microvascular Angiopoietin-1, a delayed regeneration process associated with Type 2 myofibers hypotrophy along with an elevated number of remaining cycling Pax7+ cells. In conclusion, pericytes associated with endothelial cells are essential to maintain adult muscle homeostasis and exert paracrine effects on adjacent myogenic cells during muscle repair in adulthood.

pericytes, angiopoietin-1, satellite cells, regeneration

Adult muscle regeneration- #2770

P01- 6- Role of Serum Response Factor (Srf) in muscle stem cells

Aikaterini Papaefthymiou (1), Voahangy Randrianarison-Huetz (1), Ulduz Faradova (1), Gaelle Herledan (1), Laura Collard (1), Athanassia Sotiropoulos (1)

1. Institut Cochin-Inserm U1016, Paris, France

Adult skeletal muscle can adapt its size to various stresses such as functional overload and can regenerate after injury. Muscle plasticity and homeostasis rely on satellite cells (SC) that are maintained in a quiescent state. Upon injury or increased workload, SCs are activated, divide and self-renew to replenish the niche. Proliferating myoblasts exit the cell cycle to differentiate and fuse to form new or fuse to preexisting growing muscle fibers. The project aims to investigate the mechanisms controlling the functions of adult myogenic stem cells during regeneration and overload-induced hypertrophy.

Srf transcription factor controls the expression of a wide range of genes including those involved in proliferation and myogenic differentiation. Inhibition of Srf in cultured myogenic cell lines C2C12 was shown to impede myoblast proliferation and differentiation. However data are lacking regarding the role of Srf in muscle stem cells behavior in vivo. We generated Pax7-CreERT2;Srf^{flx/flx} mice in which Srf loss is induced in SC and SC were mobilized by two means: CTX-induced regeneration and overload-induced hypertrophy. In parallel we conducted ex vivo cultures of primary myoblasts expressing or not Srf to further study the cellular and molecular processes involved.

We showed:

- At steady state, Srf null SC numbers are unchanged.
- Muscle regeneration and muscle growth are impaired in mutant muscles indicating a contribution of Srf to SC cell fate.
- Shortly after injury or overload, the numbers of SC is diminished in absence of Srf. However their proliferation capacity was unchanged. In addition Srf null SC activation seemed to be delayed (in vivo and ex vivo).
- Strikingly, early differentiation of SC was not affected by Srf loss both in vivo and in cultured SC. Accordingly MyoD and MyoG expressions were not altered by Srf loss in cultured myoblasts. In contrast, SC late differentiation and fiber fusion were compromised in vivo and in vitro by Srf loss.
- Finally, by time laps microscopy, we found that the cell motility of Srf null myoblasts is strongly decreased.

We performed transcriptomic studies and identified the set of genes whose expressions are altered by Srf loss in proliferating and differentiating myoblasts. We conducted functional studies in culture (loss of function- effect on differentiation, fusion, motility, survival) to decipher the underlying molecular mechanisms. We will present the results obtained for some candidates.

satellite cells, regeneration, hypertrophy, Srf

Adult muscle regeneration- #2864

P01- 7- A Systems Based Approach into Vitamin D and Skeletal Muscle Repair, Regeneration and Remodelling

Daniel Owens (1), Adam Sharples (2), Ioanna Polydorou (3), Nura Alwan (2), William Fraser (4), Jonathan Tang (4), James Morton (2), Claire Stewart (2), Graeme Close (2)

1. Institute of Myology, Paris, France

2. Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, Royaume Uni

3. Charité-Universitätsmedizin, Berlin, Allemagne

4. Faculty of Medicine and Health Science, Norwich Medical School, University of East Anglia, Norwich, Royaume Uni

Skeletal muscle is a direct target for Vitamin D. Observational studies suggest that low 25[OH]D correlates with functional recovery of skeletal muscle following eccentric contractions in humans and crush injury in rats [1, 2]. However, a definitive association is yet to be established.

In order to address this gap in knowledge in relation to damage repair, a randomised, placebo-controlled trial was performed in twenty males with insufficient concentrations of serum 25[OH]D (45 ± 25 nmol.L⁻¹). Prior to and following 6-weeks of supplemental Vitamin D3 (4,000 IU.day⁻¹) or placebo (50 mg cellulose), participants performed 20x10 damaging eccentric contractions of the knee extensors with peak torque measured over the following 7 days of recovery. Parallel experimentation using isolated human skeletal muscle derived myoblast cells from biopsies of 14 males with low serum 25[OH]D (37 ± 11 nmol.L⁻¹) were subjected to mechanical wound injury, which enabled corresponding in vitro studies of muscle repair, regeneration and hypertrophy in the presence and absence of 10 nmol or 100 nmol 1 α ,25[OH]2D3.

Supplemental Vitamin D3 significantly increased serum 25[OH]D and improved recovery of peak torque at 48 hours and 7 days post-exercise. In vitro, 10 nmol 1 α ,25[OH]2D3 improved muscle cell migration dynamics and resulted in improved myotube

fusion/differentiation at the biochemical, morphological and molecular level together with increased myotube hypertrophy at 7 and 10 days post-damage. Together, these preliminary data are the first to establish a role for Vitamin D in human skeletal muscle repair and suggest that maintaining serum 25[OH]D may be beneficial for optimising reparative processes and potentially for facilitating subsequent hypertrophy.

1. Barker, T., et al. *Nutrients*, 2013. 5(4): p. 1253-75.
2. Stratos, I., et al. *The Am J of Pathol*, 2013. 182(3): p. 895-904.

Vitamin D, Regeneration, Skeletal Muscle

Adult muscle regeneration- #2905

P01- 8- Pitx2 stimulates skeletal muscle repair and restores dystrophin expression and function in MDX mice

Daniel Vallejo (1), Francisco Hernández-Torres (1), Estefania Lozano-Velasco (1), Felicitas Ramirez (1), Diego Franco (1), Amelia E Aranega (1)

1. Jaén, Espagne

Pitx2 stimulates skeletal muscle repair and restores dystrophin expression and function in MDX mice.

Vallejo D., Hernández-Torres F., Lozano-Velasco E., Ramirez F., Franco D., Aranega AE.

Department of Experimental Biology, University of Jaén, Jaén, Spain.

Pitx2 is a paired-related homeobox gene that has been shown to play a role during muscle development. We have previously documented that the c-isoform of Pitx2 plays a pivotal role modulating proliferation versus differentiation during myogenesis, balancing Pax3+/Pax7+ myogenic population, and regulating key myogenic transcription factors such as Pax3 by repressing miR-27. Recently, we have evaluated the contribution of Pitx2c in the transcriptional control of miRNAs during myogenesis. Therefore, we have identify a Pitx2-miRNA pathway that regulates cell proliferation in freshly isolated satellite cells, enhancing the Myf5+ satellite cells and thereby promoting their commitment to a myogenic cell fate. Based on these results, and considering that Pitx2 expression is significantly increased during muscle regeneration but decreased in the mouse model for Duchenne muscular dystrophy (DMDmdx mice), we have carried out an experimental approach for cell transplantation "in vivo" to test whether Pitx2 could improve the regenerative capability of satellite isolated from dystrophic muscles. Importantly, the ability to modify the regenerative capability of dystrophic cells means significant advantage for therapeutic application in humans. The results obtained so far demonstrate that cell transplantation of dystrophic satellite cells overexpressing Pitx2c 1) enhances the number of myofibers, 2) repress miR-31 reaching dystrophin restoration 3) finally improving muscle function in DMDmdx mice. All together these results place Pitx2 as new player on skeletal muscle satellite cell biology and identify unknown functions of Pitx2 during regenerative myogenesis.

Pitx2 muscle regeneration

Adult muscle regeneration- #2918

P01- 9- Laminin-111 deposition in the satellite cell niche mediates satellite cell self-renewal during skeletal muscle regeneration.

Daniele Ranaldi (1), Shantisree Sandeepani Rayagiri (1), Alexander Raven (1), Nur Izzah Farhana Mohamad Azhar (2), Olivier Lefebvre (3), Jennifer Morgan (4), Peter Zammit (5), Anne-Gaëlle Borycki (1)

1. Department of Biomedical Science, University of Sheffield, Sheffield,

2. Department of Biomedical Science, University of Sheffield, Sheffield, Royaume Uni

3. Inserm U1113, MN3T team, Strasbourg, France

4. The Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, Royaume Uni

5. Randall Division of Cell and Molecular Biophysics, King's College London, New Hunt's House, London, Royaume Uni

Satellite cells are skeletal muscle-specific stem cells that are responsible for the homeostasis and repair of the skeletal muscle tissue. Satellite cells are usually quiescent, but are activated upon injury, enter the cell cycle, and differentiate, in a process reminiscent of embryonic myogenesis, to regenerate damaged muscle fibres. Satellite cells are positioned in a specific anatomical compartment between the basement membrane surrounding the myofibre and the myofibre sarcolemma. This location, known as the 'niche', ensures that satellite cells are in an optimal environment to support their activity.

The niche plays a central role in the regulation of stem cell activity. However, the relationship between the basal lamina and satellite cells has not been extensively examined. We hypothesized that the basal lamina, and in particular Laminins, has a key role in the control of satellite cells and adult myogenesis. Here, we report that the basement membrane undergoes a remodelling process to incorporate Laminin-111 upon activation of satellite cells. Furthermore, we examined the requirement for Laminin-111 in satellite cell-mediated muscle repair using pharmacological and genetics approaches. Our results reveal that Laminin-111 is essential for satellite cell expansion following their activation, and for their self-renewal. Laminin-111-mediated effects dependent on the re-expression and function of Integrin $\alpha 6\beta 1$, a receptor normally down-regulated in adult muscle cells.

These data provide novel insight into the mechanisms that contribute to the control of satellite cell activity, in particular their self-renewal, and offer a possible mechanism for the known therapeutic effect of Laminin-111 treatments in animal models of congenital muscular dystrophies.

niche, laminin, satellite cells, therapeutic

Adult muscle regeneration- #2964

P01- 10- Mechanisms of impaired myogenesis in cancer-induced skeletal muscle atrophy

Domiziana Costamagna (1), Fabio Penna (2), An Zwijsen (3), Danny Huylebrouck (4), Paola Costelli (2), Maurilio Sampaolesi (5)

1. *Translational Cardiomyology Laboratory, Stem Cell Biology and Embryology Unit, Dept of Development and Regeneration, KUL University of Leuven, Leuven, Belgique*
2. *Dept of Clinical and Biological Sciences, University of Turin, Turin, Italie*
3. *Laboratory for Developmental Signaling, VIB Center for the Biology of Disease, Dept of Human Genetics, KU Leuven, Leuven, Belgique*
4. *Laboratory of Molecular Biology (Celgen), Dept of Development and Regeneration, KU Leuven University, Leuven, Belgium; Department of Cell Biology, Erasmus MC, Rotterdam, Pays-Bas*
5. *Translational Cardiomyology Laboratory, Stem Cell Biology and Embryology Unit, Dept of Development and Regeneration, KUL University of Leuven, Leuven, Belgium; Division of Human Anatomy, Dept of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy; Leuven, Belgique*

Skeletal muscle atrophy is one of the main features occurring during cancer cachexia, a syndrome that frequently causes fat and muscle weight loss and interferes with antineoplastic therapies in terminal cancer patients. Lately, cancer-induced muscle depletion seems to be the cause of an impaired myogenesis leading to accumulation of activated myogenic precursors unable to counteract muscle atrophy¹.

During skeletal muscle regeneration, several cell types interact modulating the trafficking of stimuli responsible for fiber formation. Satellite cells (SCs) and mesoangioblasts (MABs), vessel-associated stem cells expressing pericyte markers participate in the regeneration process². Recently, we demonstrated that MAB myogenic differentiation is improved blocking BMP-SMAD-signalling³. Interestingly, Smad8-LacZ MABs, isolated from a transgenic mouse model where Smad8 expression is disrupted by LacZ gene insertion⁴, are more prone to differentiate in vitro into myotubes³. Moreover, Smad8-LacZ muscles, exposed to cardiotoxin, exhibit a higher regenerative potential compared to controls, showing an increased myofiber cross sectional area.

SCs from C26-bearing mice do not exhibit any cell autonomous defect and fully differentiate into myotubes similarly to controls. In addition, the myogenic ability of MABs isolated from C26-bearing mice is comparable to controls. Finally, when MABs from cachectic muscles are injected into β -Sarcoglycan (β SG)-null mice, are able to fuse and give rise to β SG+ myofibers.

These results confirm that both SCs and MABs isolated from C26 cachectic muscles retain their myogenic differentiation potential. Further studies are needed to test if C26-Smad8-LacZ-bearing mice undergo muscle atrophy, and eventually to verify if myogenic progenitors lacking Smad8 can positively affect the atrophic phenotype.

References:

- Penna F. et al., PLoS ONE, e13604, 2010.
 Costamagna D. et al., Curr Gene Ther. 15:348-63, 2015.
 Costamagna D. et al., JMCB, [Epub], 2015.
 Arnold JM. et al., Dev Biol. 296:104-18, 2006.

cancer cachexia, muscle atrophy, muscle regeneration, SMADs

Adult muscle regeneration- #3000

P01- 11- Role of the CCAAT-transcription factor NF-Y and its splice variants in myogenic differentiation and muscle regeneration

Valentina Basile (1), Fiorenza Baruffaldi (1), Diletta Dolfini (2), Laura Ricci (1), Roberto Mantovani (2), Didier Montarras (3), Susanna Molinari (1), Carol Imbriano (1)

1. *University of Modena and Reggio Emilia, Dep. of Life Sciences, Modena, Italie*
2. *University of Milan, Dep. of Biomolecular Sciences and Biotechnology, Milan, Italie*
3. *Pasteur Institute, Department of Developmental Biology, Paris, France*

The expression of NF-YA, the DNA-binding subunit of the CCAAT-binding transcription factor NF-Y, is down-regulated in adult skeletal muscle to allow a complete terminal differentiation. The NF-YA gene encodes for two alternative splice variants, NF-YAs and NF-YAI: in human haematopoietic and mouse embryonic stem cells, the expression of NF-YAs or NF-YAI is crucial to promote proliferation or differentiation, respectively. The role of NF-YA isoforms in skeletal myogenesis and satellite cells fate has never been investigated. Here we show that both NF-YA isoforms are induced in mouse regenerating muscle and are expressed in satellite cells (SCs). The abrogation of NF-Y activity impairs both proliferation and differentiation of SCs. Forced expression of NF-YAs stimulates SCs proliferation, while NF-YAI enhances their differentiation. The same effects are observed in mouse C2C12 myoblasts, in which the two isoforms activate opposite transcriptional programs. NF-YAs upregulates genes annotated in growth factor binding, cell adhesion and extra cellular matrix Gene Ontology terms, while NF-YAI increases the transcription of genes belonging to sarcomere, muscle cell differentiation, development and muscle contraction categories. NF-YAI boosts myoblasts differentiation by up-regulating the transcription of novel NF-Y target genes, among which Mef2D, Six4 and Cdkn1C, which are known to be involved in the differentiation program. Overall, our results highlight the functional difference between NF-YA isoforms, whose modulation could be useful to improve stem cell based therapies to treat muscular dystrophy.

Transcription factor, alternative splicing, myogenic differentiation, satellite cells

Adult muscle regeneration- #3014

P01- 12- MEF2C: a transcription factor with multiple roles in adult myogenesis

Fiorenza Baruffaldi (1), Sara Badodi (1), Susanna Molinari (1), Luca De Feo (1), Valentina Basile (1), Carol Imbriano (1), Antonio Musarò (2), Carmine Nicoletti (2), Didier Montarras (3), Margaret Buckingham (3), Renata Battini (1)

1. *Modena, Italie*
2. *Roma, Italie*
3. *Paris, France*

Satellite cells (SCs) are resident muscle stem cells that mediate post-natal muscle growth, muscle regeneration and hypertrophy. Terminal differentiation of SCs is controlled by members of the Myocyte Enhancer Factor 2 (MEF2) family of transcription factors. Regulation of MEF2 function occurs at multiple levels, including alternative pre-mRNA splicing, protein phosphorylation and association with co-regulators. However, no in vivo studies have examined these regulatory mechanisms. We report that inclusion of the a1 exon in Mef2c transcripts is upregulated in proliferating mouse SCs and in the early phases of muscle regeneration and is maintained in later stages of myogenesis. Over-expression of Mef2ca1 transcripts promotes muscle regeneration and myofiber hyperthrophy and correlates with expansion of primary myoblasts ex vivo and in vivo. The pro-proliferative activity of this splice variant is mediated by phosphorylation of the Ser98 and Ser110 residues coded by exon a1 that directs interaction with the myogenic repressor PIN1. The non-phosphorylatable mutant retains the ability to induce muscle terminal differentiation and growth. MEF2Ca1-dependent hypertrophy depends on the activation of the PI3K/AKT-dependent protein synthesis pathway and on myonuclear accretion. We report that MEF2Ca1 acts as a transcriptional switch of the expression of proliferation- (Plk2, JunB), differentiation- (TnnC1) and growth-associated genes (Igf1, Igf2) depending on the phosphorylation of the PIN1 binding sites. Our results thus reveal an important role for regulatory interactions between alternative splicing and post translational modifications of a single transcription factor in the control of the multilayered regulatory programs required for adult myogenesis.

differentiation, phosphorylation, alternative splicing, transcription, regulation

Adult muscle regeneration- #3023

P01- 13- Peculiar behavior of non-myogenic cells from fibrotic muscle

Elisa Negroni (1), Mona Bensalah (1), Victorine Albert (1), Teresa Gidaro (1), Gonzalo Cordova (1), William Duddy (1), Svetlana Ghimbovschi (2), Sophie Perie (3), Jean Lacau Saint Guily (3), Françoise Chapon (4), Gillian Butler-Browne (1), Anne Bigot (1), Vincent Mouly (1), Capucine Trollet (5)

1. Sorbonne Universités UPMC Univ Paris 06, Inserm, CNRS, Centre de Recherche en Myologie, Paris, France

2. Genomics, Proteomics, and Bioinformatics Core of the Intellectual and Developmental Disabilities Research Center (IDDR) Children's National Medical Center, Washington, Etats-Unis

3. APHP, Service d'ORL et chirurgie cervicofaciale, hôpital Tenon, UPMC Université Paris 6, 4 rue de la Chine, 75020 Paris, France

4. CHU de Caen, Centre de Compétences des Pathologies Neuro-musculaires, Service de Médecine Physique et Réadaptation, Avenue de la Côte de Nacre, CS 30001, 14033 Caen, Caen, France

5. Sorbonne Universités UPMC Univ Paris 06, Inserm, CNRS, Centre de Recherche en Myologie, Paris, France

Muscular dystrophies represent a heterogeneous group of genetic diseases affecting skeletal muscle in both children and adults and characterized by skeletal muscle weakness, wasting and degeneration. These diseases, whether they involve repeated cycles of degeneration and regeneration such as in Duchenne muscular dystrophy (DMD) or not such as Oculopharyngeal muscular dystrophy (OPMD), result inexorably in skeletal muscle atrophy to which is often associated fibrosis. Fibrotic muscular substitution is a complex and not yet fully understood process characterized by excessive accumulation of collagens and extracellular matrix¹. Fibrosis can be considered as a dysregulated tissue repair response and involves several cellular and soluble effectors. Among them, fibroblasts have an increasingly appreciated role as an autocrine/paracrine source of profibrotic stimuli² associated with tissue scar formation and fibrosis, but their causal implication in dystrophic muscle progression and the underlying mechanisms remain unclear³.

OPMD is an autosomal dominant inherited late-onset degenerative muscle disorder where a small group of specific muscles (pharyngeal and eyelid) are primarily affected. DMD is the most common inherited muscular dystrophy with widespread muscle degeneration leading to progressive muscle wasting, cardiac dysfunction, respiratory failure and ultimately death. Both dystrophies are characterized by an exacerbated fibrosis^{4,5}. Here we characterized non-myogenic cells isolated from affected muscles of OPMD and DMD patients compared to control limb muscle; this includes proliferation rate and lifespan studies, transcriptomic and FACS analysis as well as xenotransplantation to decipher their exact role during muscle regeneration. We demonstrate that human non-myogenic cells from fibrotic muscle are very different from that of control muscle with a strikingly high proliferative capacity, an inhibitory effect on muscle regeneration and an exacerbated secretion of pro-fibrotic factors. The question remains whether this peculiar behavior is 'only' the consequence of the fibrotic environment where they come from or one of the causes of this fibrotic and degenerated environment.

1. Wynn, T.A. J Pathol 214, 199-210 (2008).

2. Serrano, A.L. et al. Exp Cell Res 316, 3050-8 (2010).

3. Ardite, E. et al. J Cell Biol 196, 163-75 (2012).

4. Gidaro, T. et al. J Neuropathol Exp Neurol 72, 234-243 (2013).

5. Negroni, E. et al. J Neuropathol Exp Neurol 73, 789-97 (2014).

skeletal muscle, fibrosis, regeneration, extracellular matrix, pharyngeal muscle

Adult muscle regeneration- #3025

P01- 14- The pharmaceutical blockade of activin type IIB receptor improves absolute force-generating capacity but impairs mitochondrial function in exercising mouse gastrocnemius muscle in vivo

Nelly Béchir (1), Emilie Pecchi (1), Christophe Vilmen (1), Yann Le Fur (2), Monique Bernard (2), David Bendahan (2), Benoit Giannesini (2)

1. Aix-Marseille Université, CNRS, CRMBM UMR 7339, 13385, Marseille, France, Marseille, France

2. Aix-Marseille Université, CNRS, CRMBM UMR 7339, 13385, Marseille, France, France

Background. Because it leads to a rapid and massive muscle hypertrophy, postnatal blockade of the myostatin/activin type IIB receptor (ActRIIB) is considered as a promising therapeutic strategy for counteracting muscle wasting commonly associated to

ageing (sarcopenia), neuromuscular disorders and various catabolic diseases. However, the functional consequences of this blockade remain very poorly documented under physiological condition in vivo.

Objective. The purpose of this study was to investigate totally noninvasively the impact of 8-week administration of a soluble ActRIIB signaling inhibitor (sActRIIB-Fc, also called RAP-031, Acceleron Pharma, Cambridge, MA, USA; 10 mg/kg body weight) on gastrocnemius muscle anatomy, bioenergetics and force-generating capacity in wild-type C57BL/6 3-month old mice using in vivo magnetic resonance (MR) imaging and dynamic 31-phosphorus MR spectroscopy (31P-MRS).

Results. Compared to vehicle (PBS) control, sActRIIB-Fc treatment resulted in a dramatic increase in body weight (+29%) and muscle volume (+58%) calculated from hindlimb MR imaging, but did not alter fiber-type distribution determined via myosin heavy chain isoforms analysis. In resting muscle, sActRIIB-Fc treatment induced acidosis and PCr depletion, thereby suggesting reduced tissue oxygenation. During an in vivo fatiguing exercise (6 minutes of repeated maximal isometric contractions electrically induced at a frequency of 1.7 Hz), maximal and total absolute forces were larger in sActRIIB-Fc treated animals (+26% and +12%, respectively) whereas specific force and fatigue resistance were lower (-30% and -37%, respectively). Treatment with sActRIIB-Fc further decreased the rate of oxidative ATP synthesis (-34%) and the maximal mitochondrial capacity (-44%), but did not alter the bioenergetics status in contracting muscle.

Conclusion. Our findings demonstrate under physiological condition in vivo that sActRIIB-Fc administration increases absolute force-generating capacity but impairs mitochondrial function possibly via limitation of oxygen supply. Nevertheless, this impairment does not compromise the bioenergetics status during sustained muscle activity. Overall, these data support the clinical interest of postnatal ActRIIB blockade.

Skeletal muscle hypertrophy, fatigue, muscle weakness

Muscle regeneration- #3107

P01- 15- Use of the six-minute walk distance (6MWD) across Duchenne muscular dystrophy (DMD) studies

Michelle Parrish (1)

1. Communications, PharmaGenesis, Newtown, Etats-Unis

Abstract for Myology 2016.

Muscle regeneration- #2967

P01- 16- Muscle patterning in the regenerating axolotl tail.

Wouter Masselink (1), Carr Vincent (1), Elly Tanaka (1)

1. Dresden, Allemagne

Wouter Masselink1, Carr Vincent1, Elly Tanaka1

1. Center for Regenerative Therapies Dresden- CRTD, TU Dresden, Germany

Ambystoma mexicanum (axolotl) is a species of neotenic aquatic salamanders that have extensive regenerative capacity. Axolotl continue to grow throughout their life and exhibit extensive regenerative capacity. Interestingly the tail not only expands in size, but also exhibits formation of de-novo myogenic elements. Similar segmentation is also apparent during regeneration. While during embryogenesis the somitic clock predominantly specifies this process, during both regeneration and non-embryonic growth it seemingly proceeds without any apparent formation of somites.

We hypothesise myotome segmentation during non-embryonic stages and in regeneration to be fully dependent on the recruitment of adaxial cells. During zebrafish embryogenesis adaxial cells which have their origin in the medial part of the somite adjacent to the notochord. Hedgehog signaling from the notochord to the adaxial cells induces their lateral migration and eventual formation of slow muscle fibers. However, incorrect adaxial cell specification by the inhibition of hedgehog signaling results in the formation of atypical vertical myosepta and the complete absence of horizontal myosepta.

Preliminary evidence suggests adaxial cells are also present in Axolotl. Furthermore, axolotl exposed to cyclopamine (a potent inhibitor of the hedgehog signaling pathway) during tail regeneration exhibit a disruption in the re-patterning of myotomal segments. Since no prior segmentation pattern exists, this would imply segmentation and thus myofiber length is ultimately determined autonomously by the myofiber. We are currently undertaking analysis of myofiber length and myotome boundary formation to elucidate the morphogenetic changes underlying this process.

Axolotl, muscle, segmentation, patterning, adaxial cells.

Muscle regeneration- #3243

P01- 17- TGF β 1 PATHWAY IN RAT SKELETAL MUSCLE REGENERATION

Paulina Kasprzycka (1), Wladyslawa Streminska (1), Katarzyna Ilach (1), Maria Anna Ciemerych (1), Malgorzata Zimowska (1)

1. Department of Cytology, Faculty of Biology, University of Warsaw, Warsaw, Pologne

Injured skeletal muscles, which are a mosaic of fast and slow fibers, are able to regenerate. Repair of fast twitch muscles, such as EDL (95% fast fibres), proceeds properly leading to the reconstruction of functional tissue. However, in some muscle types, i.e. slow twitch muscles, such as Soleus muscle (85- 100 % slow fibres), repair is often inefficient and might be affected by the development of fibrosis. The significant role in muscle regeneration is played by muscle-specific stem cells, i.e. satellite cells. In the response to the injury satellite cells become activated, proliferate, fuse to myotubes, and reconstruct destroyed muscle fibers. Next, the extracellular components contribute to successful muscle repair. Muscle repair is controlled by various factors: cytokines, growth factors, such as TGF β 1, and also enzymes impacting at extracellular matrix (ECM). Among such enzymes are matrix metalloproteases (MMP). Two of them, i.e. MMP-2 and MMP-9, were shown to be characteristic for skeletal muscles and involved in its regeneration. Next, TGF β 1- a multifunctional cytokine- was shown to be involved in the regulation of fibrosis development. TGF β family members are well known as stimulators of ECM production and modulators of the expression of

ECM degrading enzymes and proteinase inhibitors. TGF β 1 also mediates MMP-2 and MMP-9 activation. In our current study we tested the interaction between TGF β 1 and MMP-2, MMP-9 in slow and fast twitch muscle regeneration and also in in vitro differentiating myoblasts isolated from these muscles.

skeletal muscle, myoblast, regeneration, differentiation, TGF β 1, MMP-9, MMP-2

P02- ALS and other motor neuron diseases (except SMA)- N° 18 to N° 26

ALS and other motor neuron diseases (except SMA)- #2299

P02- 18- Genetics of Amyotrophic Lateral Sclerosis (ALS) disease in Iran

Afagh Alavi (1), Elahe Elahi (1), Shahriar Nafissi (2), Mohammad Rohani (3), Hosein Shamshiri (2)

1. No, School of Biology, College of Science, University of Tehran, Tehran, Iran, Tehran, Iran

2. No, Department of Neurology, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran

3. No, Department of Neurology, Iran University of Medical Sciences, Hazrat Rasool Hospital, Tehran, Iran, Tehran, Iran

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease. It is generally adult onset and fatal. ALS usually appears sporadic (SALS), but 1%-13% of cases are familial (FALS). Today, more than 20 causative genes are known, and mutations in most are very rare. Based on functions of the genes, oxidative stress, axonal transport, vesicular transport, protein aggregation, and RNA metabolism are relevant to ALS pathology. Importantly, mutations in various ALS genes have also been observed in other neurodegenerative diseases and it is thought that understanding the pathology of ALS will contribute to understandings of other disorders. We for the first time studied the genetic basis of ALS in 107 Iranian patients. Average age at onset in our cohort was relatively young and survival was long. Linkage analysis in four families using high density SNP microarrays led to identification of a locus that included the well-known SOD1 gene in three of the families. Mutation screening identified the common p.Asp90Ala mutation in SOD1 in the three families. Subsequent screening of SOD1 in all patients revealed it is causative in approximately 12% of the cohort, and in 35% of the FALS cases. The hexanucleotide repeat expansion mutation in the C9ORF72 gene and mutations in TARDBP gene were screened in the Iranian patients by repeat-primed PCR genotyping and direct sequencing, respectively. The expansion was observed in only two patients, and TARDBP mutation was detected only in one patient, suggesting that compared to most Western populations wherein hexanucleotide expansion is the most important gene, its contribution to ALS in Iran is notably less. As in most populations, mutations in TARDBP were very rare.

Amyotrophic Lateral Sclerosis, ALS, SOD1, C9ORF72, TARDBP

ALS and other motor neuron diseases (except SMA)- #2310

P02- 19- Demographic and clinical features of ALS in northeastern Iran from March 2007 through March 2013; A case series study

Reza Boostani (1), Behnaz Khazaei (1)

1. Mashhad, Iran

Objectives: Only few studies have been performed on the epidemiology and clinical aspects of ALS in the Middle East. This study aims at such, in northeastern Iran.

Methods: Demographic and clinical data of ALS patients in Khorasan province (pre-2004) were gathered and analyzed.

Results: Of 71 patients, 59 fulfilled El Escorial Criteria and entered the study with a significant male preponderance (ratio: 1.8- p: 0.027). Personal and medical histories, electromyography reports (EMGs), and initial and final clinical presentations were gathered in detail. Considering the mean age of onset (47.7 years) for dividing the patients into two groups, there was no significant difference between them regarding sex, onset region and UMN/LMN pattern of onset whereas there was a significant one in disease duration of deceased patients (as a surrogate value for survival) (p: .042). Disease duration of deceased patients significantly correlated with symptom onset-diagnosis interval (p: .009- r: .670) and symptom onset-tracheostomy interval (p: .025- r: .868).

Conclusion: Mean age in our study was less than most previous studies; however, the clinical pattern was similar. Disease duration of deceased patients correlated with age at onset, symptom-diagnosis interval and symptom-tracheostomy interval.

Amyotrophic lateral sclerosis, upper motor neuron, lower motor neuron, demographic, clinical pattern

ALS and other motor neuron diseases (except SMA)- #2488

P02- 20- Spg11 knockout mouse, a model of slowly progressive Amyotrophic Lateral Sclerosis

Julien Branchu (1), Laura Sourd (1), Alexandrine Corriger (1), Esteves Typhaine (1), Raphaël Matusiak (1), Maxime Boutry (1),

Khalid Hamid El Hachimi (1), Magali Dumont (1), Alexis Brice (1), Giovanni Stevanin (1), Frédéric Darios (1)

1. Inserm U1127, CNRS UMR7225, UPMC UMR_S975, Institut du Cerveau et de la Moelle épinière, Paris, France

Motor neuron diseases regroup a variety of neurological disorders with overlapping phenotypes. Among these diseases, Amyotrophic Lateral Sclerosis (ALS) is the most frequent and is characterized by spasticity, muscle weakness and wasting. The symptoms generally progress rapidly leading to death within 3 to 5 years due to the loss of both upper and lower motor neurons. Hereditary Spastic Paraplegias (HSP) constitute the second most frequent group of motor neuron diseases characterized by progressive bilateral weakness, spasticity and loss of vibratory sense in the lower limbs. These symptoms are mainly due to the degeneration of axons of the upper motor neurons of the corticospinal tract. The clinical overlap between these pathologies is supported by the mutations found in the SPG11 gene, which are the main causes of autosomal recessive HSP and are also responsible for slowly progressive autosomal recessive ALS with juvenile-onset.